CORRESPONDENCE

First identification of VIM-4 metallo-β-lactamase in *Acinetobacter* spp.

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We read with interest the study in *CMI* by Wroblewska *et al.* [1], which reported the identification of a VIM-type metallo-β-lactamase (MBL) determinant in *Acinetobacter baumannii* in Poland. Resistance to carbapenems in *Acinetobacter* spp. is increasingly reported worldwide and is associated with various resistance mechanisms, but mostly with the expression of carbapenemases [2]. Carbapenem-hydrolysing class D β-lactamases are widespread and have been identified worldwide in *Acinetobacter* spp., whereas MBLs are much less prevalent in this genus [3]. VIM-2-producing *Acinetobacter* spp. have been isolated in the Far East [4] and in Germany [5], while the VIM-1 determinant has been reported only once in *A. baumannii* in Greece [6]. VIM-4 is a point mutant of VIM-1 and has previously been identified only in Enterobacteriaceae [7] and *Pseudomonas* spp. [8]. We now wish to report the identification of the VIM-4 MBL determinant in *Acinetobacter* genomospecies 16.

Twenty-two carbapenem-resistant clinical isolates of *Acinetobacter* spp. were collected between 2001 and 2006 at the Papageorgiou General Hospital, Thessaloniki, Greece. Since there is an increasing incidence of MBL determinants among enterobacterial [9] and *Pseudomonas aeruginosa* isolates in Greece [10], we aimed to evaluate their possible spread among *Acinetobacter* spp. The isolates were characterised using phenotypic (ATB 32 GN and Vitek 2 systems; bioMérieux, Marcy-l'Etoile, France) and genotypic (16S rRNA gene sequencing [11]) approaches. Imipenem susceptibility was determined using the disk-diffusion technique (http://www.sfm.fr) and antibiotic disks from Sanofi-Diagnostics Pasteur (Marnes-La-Coquette, France). The production of MBL was evaluated using the imipenem-EDTA Etest synergy test as recommended by the manufacturer (AB Biodisk, Solna, Sweden). A single isolate, *Acinetobacter* sp. 154, yielded a positive MBL test. This isolate was resistant to ceftazidime (MIC 256 mg/L), imipenem (MIC 32 mg/L) and meropenem (MIC 32 mg/L), but was susceptible to amoxycillin–clavulanate and ticarcillin–clavulanate combinations, and also to aztreonam. In addition, isolate 154 was resistant to chloramphenicol, sulphonamides, amikacin and kanamycin, but was susceptible to gentamicin, nalidixic acid, fluoroquinolones, rifampicin and tetracycline. Production of extended-spectrum β-lactamase was evaluated using a double-disk synergy test [12], with negative results.

Isolate 154 was from the sputum of a patient aged 80 years who was hospitalised in the internal medicine ward. Whole-cell DNA of *Acinetobacter* sp. isolate 154 was tested by PCR using primers specific for bla_{IMP} and bla_{VIM} [13], giving rise to a bla_{VIM}-positive amplicon. Sequencing performed using a GeneAmp PCR system 9700 (AB Applied Biosystems, Foster City, CA, USA) revealed that isolate 154 possessed bla_{VIM-4}. PCR combinations using the 5′-CS, 3′-CS and bla_{VIM} primers [14] revealed that bla_{VIM-4} was part of a class 1 integron, being the second gene cassette after the *aacA4* gene encoding the AAC(6′)-Ib acetyltransferase. In order to determine the exact location of the bla_{VIM-4}-containing integron, an I-*Ceu*I-restriction experiment was performed [15]; this revealed that the bla_{VIM-4} gene was chromosomally located (Fig. 1). No additional bla_{OXA-23}, bla_{OXA-40}, bla_{OXA-58} or bla_{OXA-51}-type carbapenemase genes were present in isolate 154 according to the results of PCR assays [16].

Precise identification of isolate 154 to the species level using a genotypic approach revealed that this strain belonged to *Acinetobacter* genomospecies 16, and not to *A. baumannii*, as was suggested by initial identification results based on the use of biochemical techniques. This result was in accord with the failure to detect the intrinsic $bla_{OXA-51-like}$ gene of *A. baumannii* in this isolate.

In conclusion, this is the first report of the identification of the MBL VIM-4 determinant in *Acinetobacter* spp., which emphasises the fact that carbapenem-hydrolysing class D β-lactamases are not the sole factor leading to the emergence of resistance to carbapenems in this genus. Interestingly, bla_{VIM-4} was identified in a non-*A. baumannii* isolate, thereby indicating that non-clinically significant Gram-negative species may also be reservoirs of MBL-encoding genes. Also noteworthy is the occurrence of VIM-4 in *Acinetobacter* in a country in which VIM-4 has been reported previously in *P. aeruginosa*. Thus, as observed previously with bla_{VIM-1} in Greece, this is yet another example of resistance genes known to be widespread in *P. aeruginosa* apparently crossing the species barrier to reach *Acinetobacter* spp.

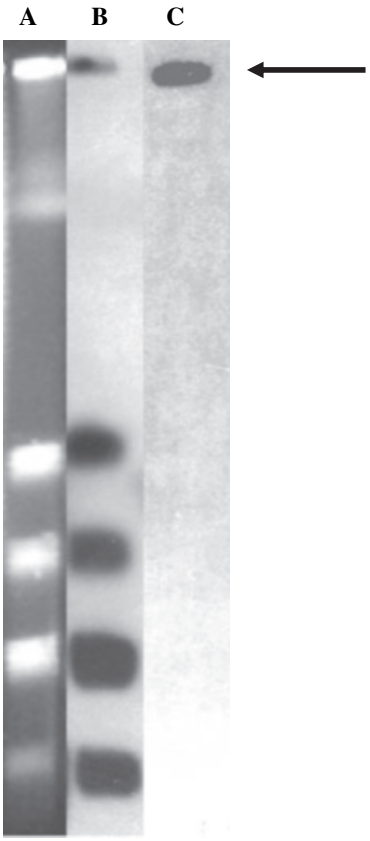

Fig. 1. (A) Pulsed-field gel electrophoresis profile of I-*Ceu*I-digested whole-cell DNA of *Acinetobacter* genomospecies 16 isolate 154. Southern hybridization was performed with a 16S–23S rRNA gene-specific probe (B) and a bla_{VIM}-specific internal probe (C). The arrow indicates the band for which a co-hybridisation signal was obtained.

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